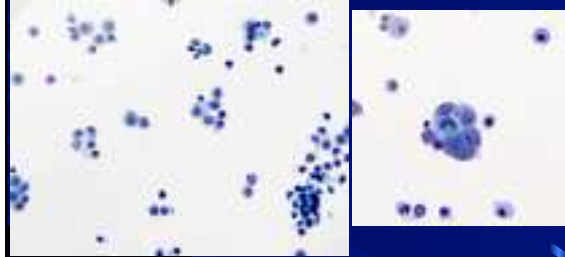


Effusion Cytology: Diagnostic Challenges

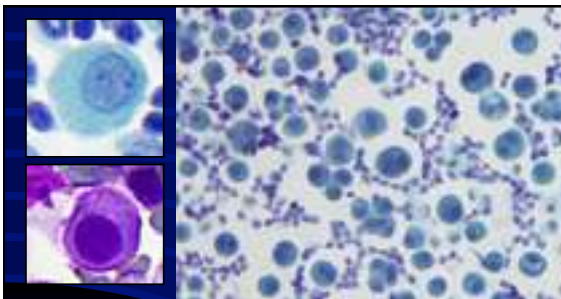
Tarik M. Elsheikh, MD

Medical Director, Anatomic Pathology
Cleveland Clinic Laboratories

Benign Mesothelial Cells



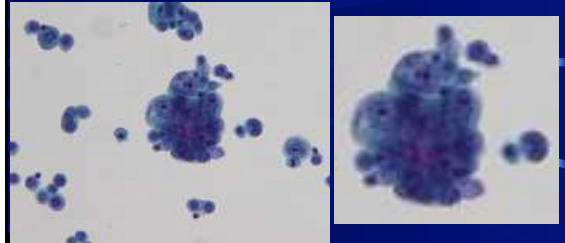
- Variable cellularity, mostly single cells
- Occasional small clusters, < 10-12 cells
 - Exception: washings
- Windows



Spectrum of change

- Bi-nucleation and multi-nucleation common
- Dense cytoplasm, clear outer rim (lacy skirt)
- Oval-round nuclei, pale chromatin
- Small nucleoli, may be prominent

General Features of Malignancy



- **Second (foreign) population**
 - Distinctly different from mesothelial cells
 - Cohesive clusters, > 10-12 cells
- **Malignant nuclear features**

CAP Inter-laboratory Comparison Program in Body Cavity Fluids

- Data bank of 10,396 lab responses, 1997-2001
- Assessed characteristics of specimens that performed well and performed poor
- Poor performers in *hypocellular* and *hypercellular* malignant specimens

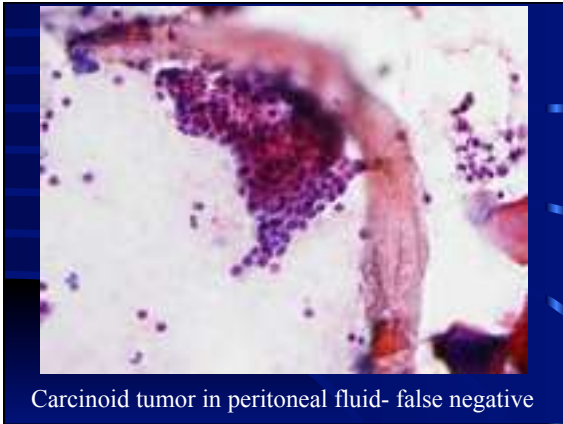
Moriarty et al 2004

CAP inter-laboratory Comparison Program ²

Hypocellular: Location errors

- Rare malignant cells, had diagnostic features but were overlooked.
- Adeno ca, squamous ca, small cell ca, melanoma and lymphoma
- This emphasizes importance of careful methodical screening

Moriarty et al 2004



CAP inter-laboratory Comparison Program ³

Hypercellular: Interpretation errors
Cellularity not a factor

- Poor carcinoma performers
 - Single cells
 - 3-D clusters
- Poor mesothelioma and lymphoma performers

Moriarty et al 2004

Common Challenges in Fluids

- Atypical cells, suspicious but not diagnostic of malignancy
- Obviously malignant cells, but uncertain classification or site of origin

Atypia in Reactive Mesothelial Cells

- Nuclear hyperchromasia
- High N/C ratio
- Prominent nucleoli

- Definitely malignant- difficult to distinguish mesothelioma from CA
- Overlapping morphologic features

OUTLINE

- Immunohistochemistry
- Cytomorphologic features
 - Specific histologic types
 - Architectural patterns
 - Potential pitfalls

Immunohistochemistry

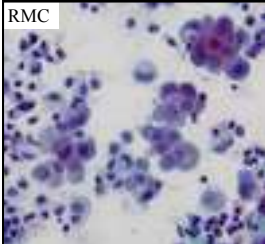
- PD malignancy → specific cell lineage
- Determine primary site
 - Differential cytokeratins and non-keratins
 - Organ specific markers
- Adeno CA vs. reactive mesothelial cells or mesothelioma

Practical IHC Panel

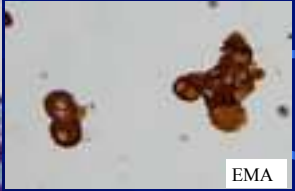
	Adeno CA	Mesothelial
Ber-EP4, MOC-31, B72.3	+	-
Calretinin, WT1, CK5/6, D2-40	-	+
TTF-1, Napsin A (lung)	+	-
mCEA	+	-
Leu-M1 (CD 15)*	+	-
EMA**	+	+/-

* CD15: good marker in tissue, BUT in fluids, also stains inflammatory cells → interpretation compromised

RMC



IHC- issues



EMA

- EMA + in most adenoca and mesotheliomas
 - Thick membrane + in mesothelioma
 - Diffuse cyto + in adeno ca
- Neg in reactive mesothelial cells (+ in 4% of cases)
- **Antibody dependent and Lab dependent**

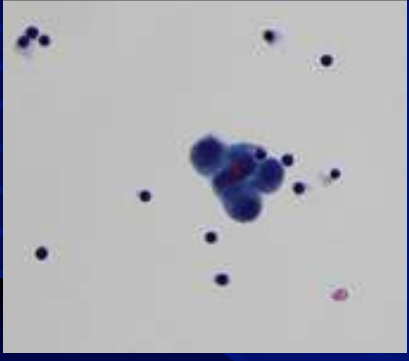
EMA: Antibody Dependent

Table 3 Sensitivity, specificity and false-positive rate of anti-EMA antibodies assessed by immunocytoLOGY on confirmed mesothelioma effusions and cases of benign reactive effusions (*Creaney 2008*)

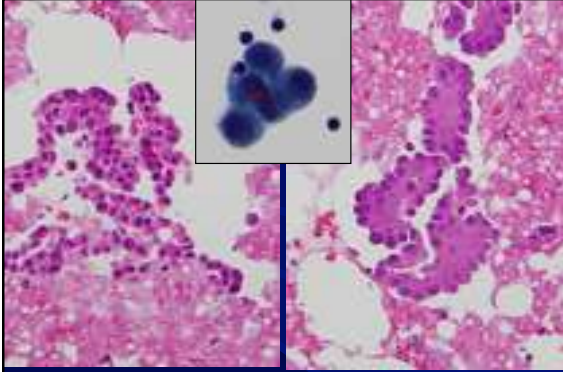
Anti-EMA clone	Sensitivity	Specificity	False positives
VU257	5/20 (25%)	16/16 (100%)	0%
VU4H5	5/20 (25%)	16/16 (100%)	0%
CDL367 (VU10Ca)	6/19 (32%)	15/15 (100%)	0%
MA552	9/18 (50%)	12/13 (92%)	0%
MA495	14/20 (70%)	5/15 (33%)	0%
E29	16/19 (84%)	14/15 (93%)	0%
Mc5	20/20 (100%)	0/14 (0%)	8/14 (57%)

- Clone E 29 (Dako) has best S&S for mesothelioma
- Clone Mc5 is not reliable to differentiate MM from RMC

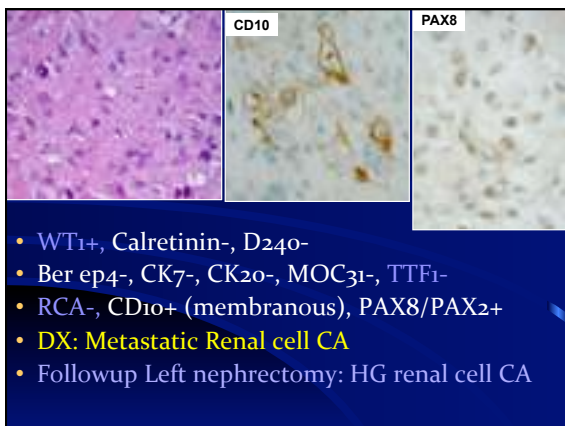
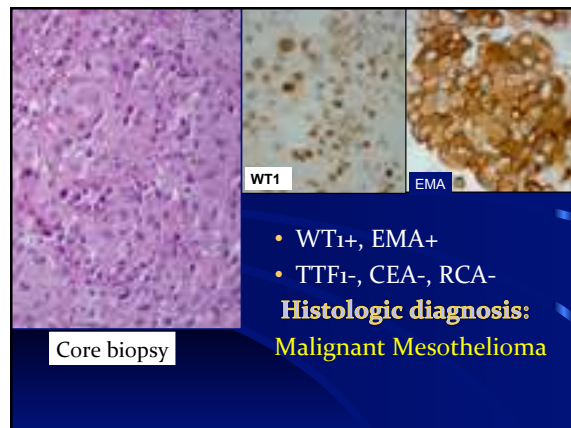
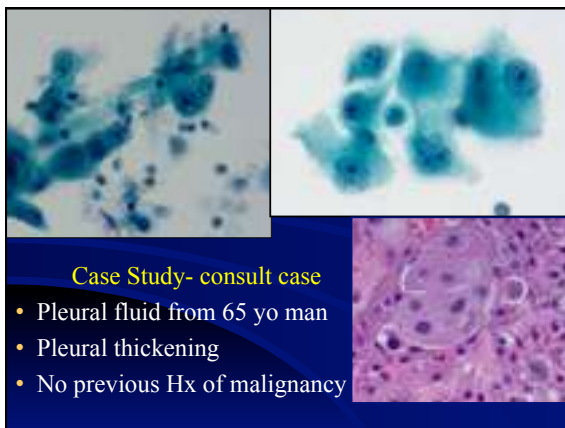
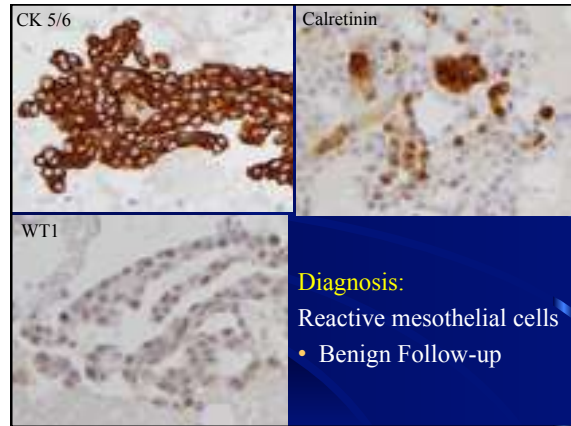
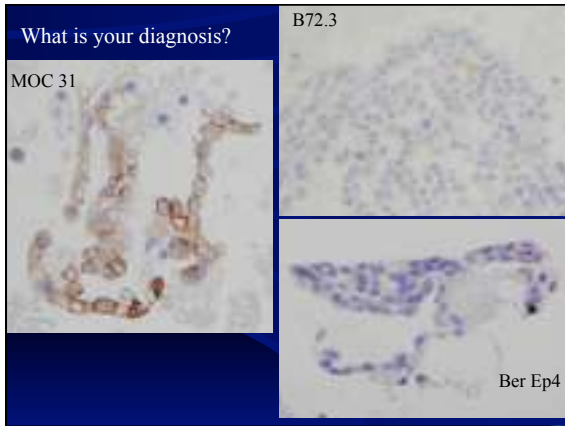
Saad 2005, Leong 2006, Creaney 2008



Case Study: Pleural fluid from 52 yo man



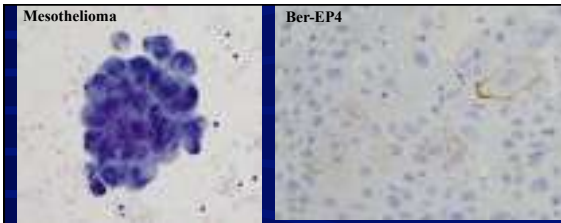
- Diff DX: Reactive mesothelial cells vs. Adenocarcinoma



Renal Cell CA vs. Mesothelioma

- Clinically, pleural metastases of RCC can resemble MM:
 - Pleural masses, nodular pleural thickening, or diffuse pleurotrophic growth with encasement of the lung
- IHC:
 - Calrtenin & WT1 positive in up to 10% of RCC
 - RCCA & CD10 positive in 8-50% of MM
- PAX8 is most sensitive and specific marker for RCC

MazalPR, et al., Mod Pathol 18:535-40, 2005



• Many neoplasms show overlapping immunoreactivity

- Mesothelial } • Calretinin + → 10-30% adenoca
- } • D2-40 + → 15% serous ca
- Epithelial } • MOC 31 + → 10% mesothelioma
- } • Ber-EP4 + → 0-30% mesothelioma

Squamous CA vs. Mesothelioma

- Overlapping Reactivity in IHC

	Mesothelioma	Squamous CA
Calretinin	+ 100%	+ 30 %
D2-40	+ > 90%	+ 50 %
CK 5/6	+ > 90%	+ 100 %
MOC31,CEA,B72.3	(-) [+ < 10%, focal]	(+) 50-95 %
WT1*	(+) > 90%	(-) 0 %
P63* (? P40)	(-) 0 %	(+) 100 %

IHC issues

- Calretinin is sensitive for mesothelioma, but not specific
- Epithelial markers are useful in distinguishing mesothelioma from carcinoma, but not squamous ca from adeno ca
- IHC panel should include positive and negative markers for each possible DX
 - Optimum 2 positive and 2 negative markers

Cytomorphologic Features

Malignant effusions


- Rarely present as an occult malignancy (7-14%)
- Most patients have established history of malignancy
- Poor prognostic sign
- Lung, breast, ovary, GI tract- most common

Problems in Malignant Effusions


- Cells tend to round up in fluids → a certain degree of uniformity among various cell types
 - Exaggerated in ThinPrep (personal experience)
- Mets in fluids, therefore, may not necessarily exactly resemble the primary tumor
- May lead to inaccurate classification of malignancy
- Familiar with different presentations of specific histologic types

Adenocarcinoma


- Most common cancer in effusions
- Foreign population
- Large clusters or isolated cells
- Many architectural patterns
 - Cell balls/cannonballs
 - Signet ring
 - Papillary
 - Multinucleation
 - Acini
 - Bizarre giant cells
 - Single cells (large or small)
 - Clear cells
 - Linear/Indian file
 - Psammoma bodies



Cannonballs



Breast



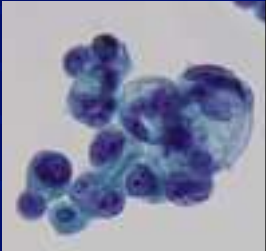
Mesothelial hyperplasia

- Tightly cohesive spherical cell clusters with an almost perfectly round contour and community border
- Uniform cells → suggest breast (more common)
- Pleomorphic cells → suggest ovary, lung
- Mesothelial hyperplasia and mesothelioma

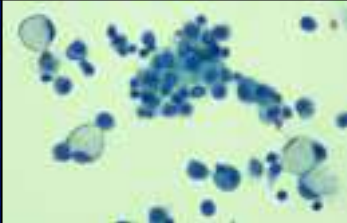
Cytoplasmic Vacuoles

Vacuoles without indentation

- Non-specific, often degenerative
 - Benign
 - Malignant: nonspecific-ovary, lung, pancreas, etc.




Signet Ring Cells




Vacuoles indent the nucleus

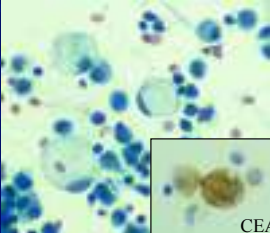
- Lobular breast carcinoma
- Gastric carcinoma
- Colon



Diff DX: Degenerative Changes





Benign



CEA

- Degenerated mesothelial cells and histiocytes can have large vacuoles imparting a signet ring appearance

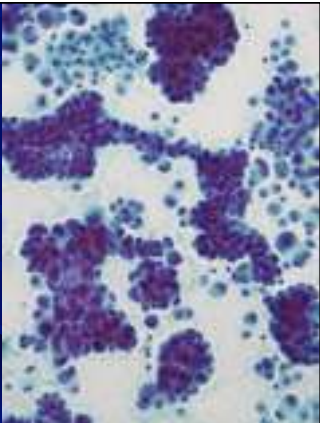
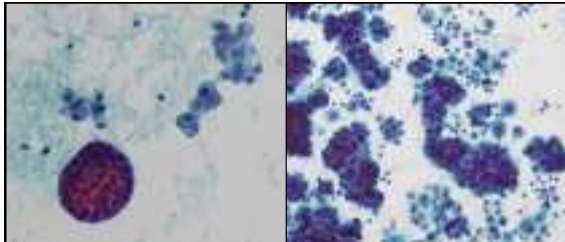
Acinar groups


- 3D groups with lumens
- Better appreciated on cell blocks
- Characteristic of adeno ca, but not specific
 - Lung, colon, ovary, stomach, breast, etc.
 - Mesothelial hyperplasia, mesothelioma
 - Small round cell tumors

Papillary

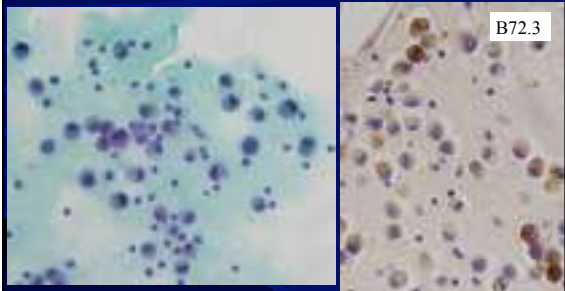
- 3-D clusters that are longer than wider
- Ascites → ovary, uterus
- Pleural effusions → lung, breast
- Others: GU, pancreas, 1° peritoneal, mesothelial hyperplasia, mesothelioma

- Gastric and colo-rectal cancers:
 - May lose tall columnar configuration in fluids
 - Cannonball, papillary

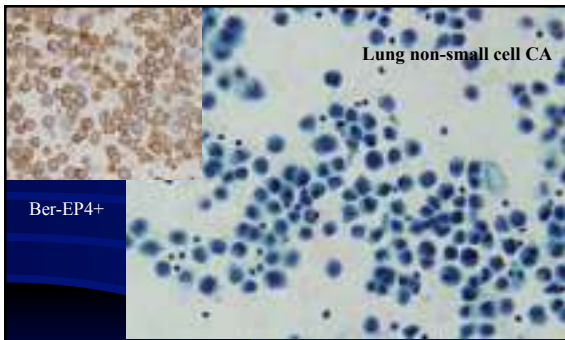


Pitfalls- Adeno CA



- Tumor cells resemble histiocytes
- Single large cells

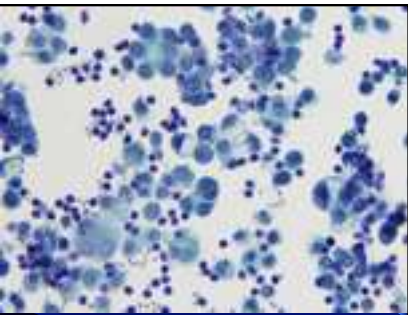
Lung non-small cell CA



Ber-EP4+

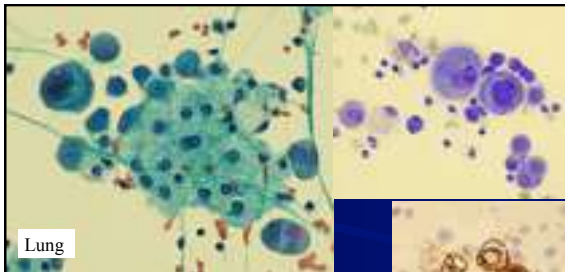
- A second population of benign mesothelial cells may be absent

Potential Pitfalls



- Breast
 - Relatively common: predominance of intermediate cells with bland features, hidden among mesothelial cells → potential false negative diagnosis

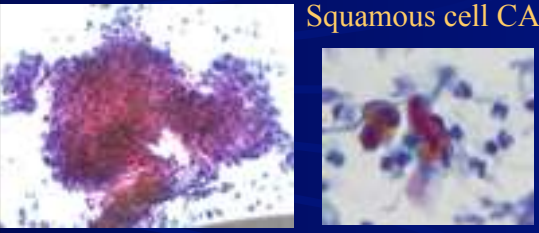
Lung




- Tumor cells may resemble mesothelial cells
- Lobular CA, lung, ovary, melanoma
- IHC helpful in these situations

MOC31

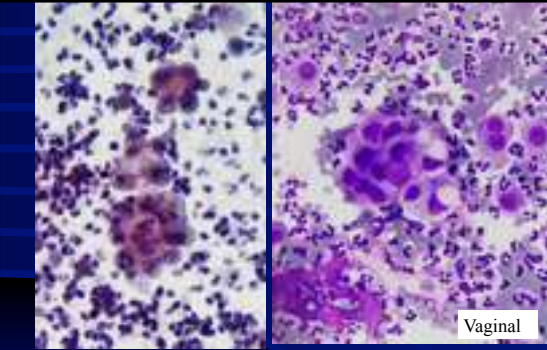
Squamous cell CA



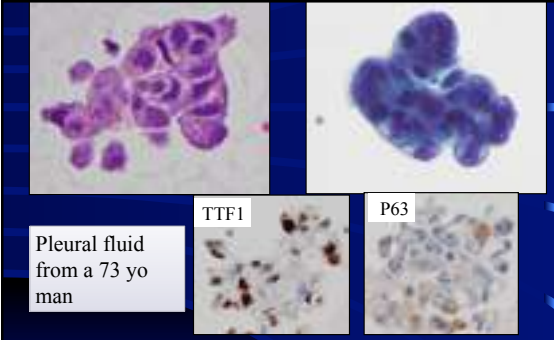
- < 1% of effusions contain squamous ca
- Primary tumor is known in most cases
- Most commonly from lung, larynx and GYN
- Single cells or clusters, dense cytoplasm
- Keratinization is rarely seen (10%)



- Squamous Cells tend to round up in fluids and may form balls
- Maybe difficult to distinguish squamous from adeno ca

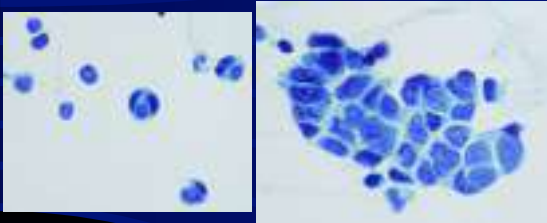


- Squamous CA frequently vacuolated
- Cytoplasmic vacuoles are nonspecific in malignancy



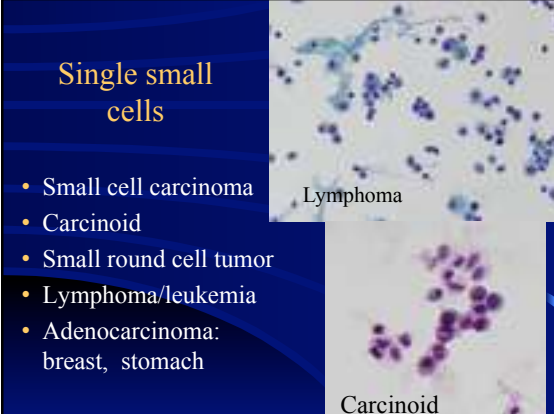
- Cell block: adeno CA may resemble squamous CA
- IHC and Pap cytology are more reliable

Small Cell Carcinoma



- Isolated cells, clusters and chains
- Round or angular nuclei, molding
- May show elongation and spindling


Single small cells




- Small cell carcinoma
- Carcinoid
- Small round cell tumor
- Lymphoma/leukemia
- Adenocarcinoma: breast, stomach

Indian/single files

- Small cell ca
- Breast
- Carcinoid tumor
- pancreas
- gastric
- mesothelioma




Small cell

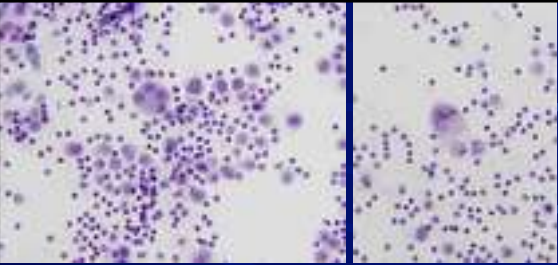


PD carcinoma

Melanoma



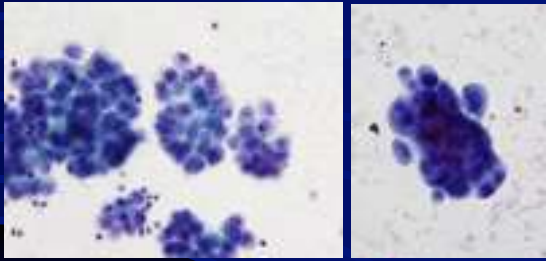
Diagnosis can be very subtle in effusions



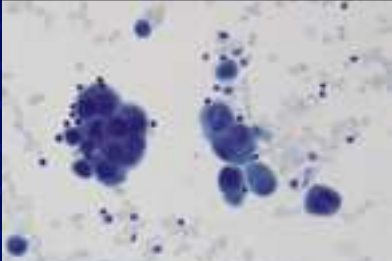
- Resemble mesothelial cells
 - isolated round cells
 - prominent nucleoli
 - clusters are uncommon
- Rarely pigment or intra-nuclear inclusions
- ICC for S100, HMB 45, Melan A

- May show single bizarre cells

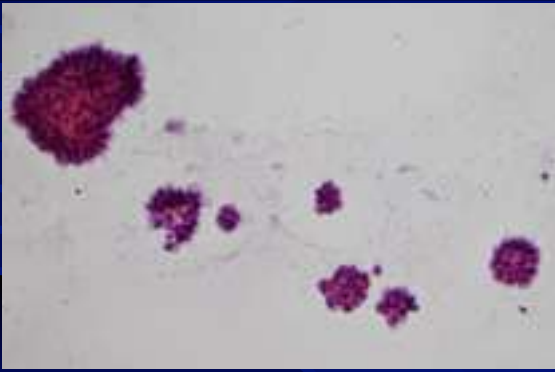
Mesothelioma



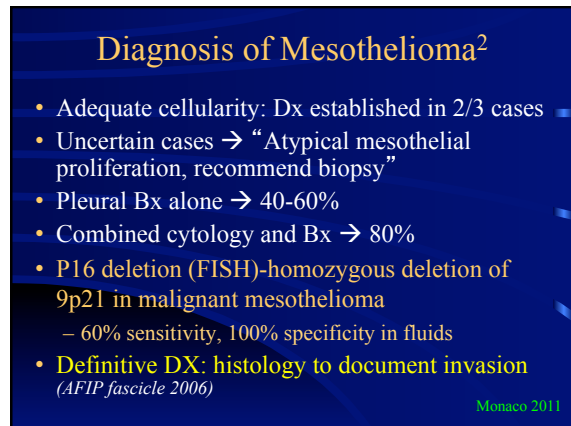
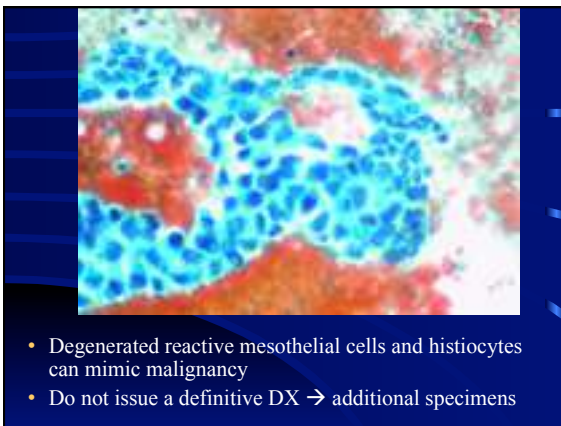
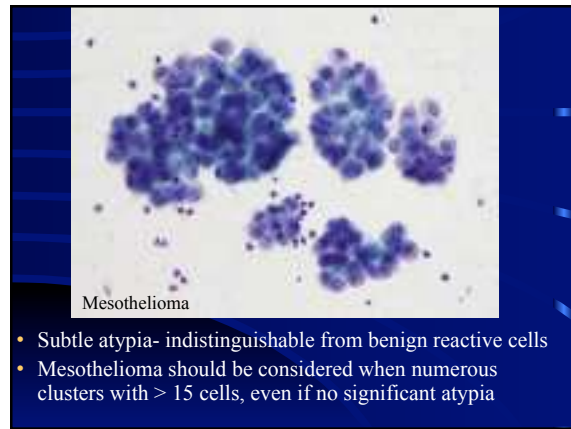
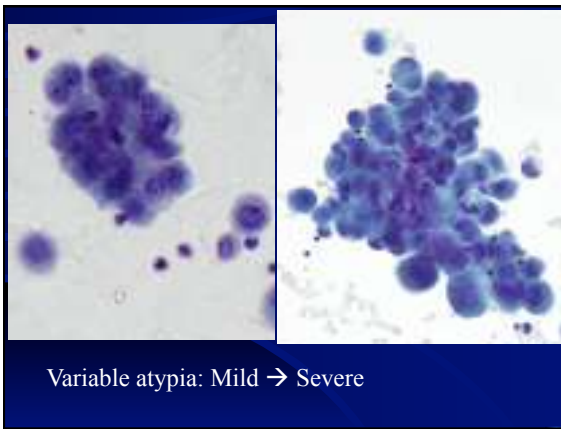
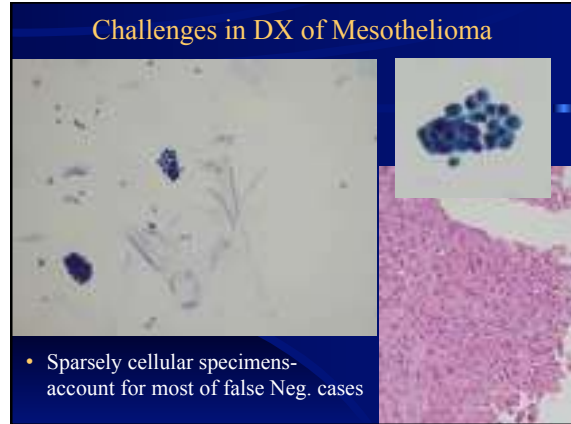
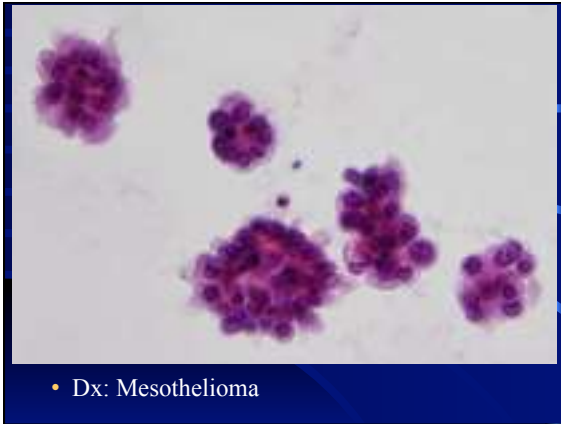
- High cellularity, large clusters 30-200 cells
- Clusters have irregular knobby borders



- Mesothelial appearance of cells
- Spectrum: benign → atypical → malignant (No second foreign population)
- Single cells may predominate



- Pleural fluid, 66 yo man



Summary

- Careful methodical screening to eliminate location errors
- Appreciation of low power architectural patterns in addition to high power cytologic details
- Awareness of diagnostic challenges and potential pitfalls
- IHC is a powerful tool, but need to be familiar with overlapping reactivity patterns



Thank You